




09/509188
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FORM PTO-1390 (Modified) (REV 5-93)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				065691/0184	
				U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) Unassigned	
INTERNATIONAL APPLICATION NO. PCT/FR98/02042		INTERNATIONAL FILING DATE September 23, 1998		PRIORITY DATE CLAIMED September 23, 1997	
TITLE OF INVENTION MICROSPORE-SPECIFIC PROMOTER AND METHOD FOR PRODUCING HYBRID PLANTS					
APPLICANT(S) FOR DO/EO/US Jan DROUAUD, Agnes FOURGOUX, Georges PELLETIER and Philippe GUERCHE					
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:					
1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.					
2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.					
3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).					
4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 th month from the earliest claimed priority date.					
5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)					
6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).					
7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made.					
8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).					
9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).					
10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).					
Items 11. to 16. below concern other document(s) or information included:					
11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.					
12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.					
13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.					
14. <input type="checkbox"/> A substitute specification.					
15. <input type="checkbox"/> A change of power of attorney and/or address letter.					
16. <input checked="" type="checkbox"/> Other items or information: Copy of Sequence Listing (4 pgs)					

U.S. APPLICATION NO. (if known, see 37 CFR 1.55) Unassigned 09/509188		INTERNATIONAL APPLICATION NO. PCT/FR98/02042		ATTORNEY'S DOCKET NUMBER 065691/0184	
17. <input checked="" type="checkbox"/> The following fees are submitted:				CALCULATIONS	
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO\$840.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482).....\$670.00					
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))\$690.00					
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO\$970.00					
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)\$96.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$840.00	
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))					
Claims	Number Filed	Included in Basic Fee	Extra Claims	Rate	
Total Claims	34	20	14	\$18.00	\$252.00
Independent Claims	3	3	0	\$78.00	\$0.00
Multiple dependent claim(s) (if applicable)				\$260.00	\$260.00
TOTAL OF ABOVE CALCULATIONS =				\$1352.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$0.00	
SUBTOTAL =				\$1352.00	
Processing fee of \$130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f)).				+	
TOTAL NATIONAL FEE =				\$1352.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +					
TOTAL FEES ENCLOSED =				\$1352.00	
				Amount to be: refunded \$	
				charged \$	
<p>a. <input checked="" type="checkbox"/> A check in the amount of \$1352.00 to cover the above fees is enclosed.</p> <p>b. <input type="checkbox"/> Please charge my Deposit Account No. <u>19-0741</u> in the amount of \$1352.00 to the above fees. A duplicate copy of this sheet is enclosed.</p> <p>c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0741</u>. A duplicate copy of this sheet is enclosed.</p>					
<p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p>					
<p>SEND ALL CORRESPONDENCE TO:</p> <p>Foley & Lardner Washington Harbour 3000 K Street, N.W., Suite 500 Washington, D.C. 20007-5109</p>				<p> SIGNATURE</p> <p>NAME PATRICIA D. GRANADOS</p> <p>REGISTRATION NUMBER 33,683</p>	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application No. :

U.S. National Serial No. :

Filed :

PCT International Application No. : PCT/FR98/02042

VERIFICATION OF A TRANSLATION

I, the below named translator, hereby declare that:

My name and post office address are as stated below;

That I am knowledgeable in the French language in which the below identified international application was filed, and that, to the best of my knowledge and belief, the English translation of the international application No. PCT/FR98/02042 is a true and complete translation of the above identified international application as filed.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the patent application issued thereon.



Date: 22 February 2000

Full name of the translator :

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MICROSPORE-SPECIFIC PROMOTER AND METHOD FOR PRODUCING
HYBRID PLANTS

The present invention concerns in particular a
5 microspore-specific promoter and a method for producing
hybrid plants.

The microspore corresponds to a precise stage
in the development of the male gamete in higher plants.
Male gametogenesis takes place in a specialized organ,
10 the anther, and comprises *sensu stricto* the
differentiation of diploid cells into haploid pollen
grains. Each diploid cell, called a sporogenic cell,
undergoes meiosis to produce four haploid microspores
which subsequently differentiate to give mature pollen
15 grains.

Knowing the molecular factors which control the
development of the microspore, and how to manipulate
them, is a considerable asset not only from a
fundamental research point of view but also from a
20 plant-improvement point of view. This is because this
knowledge enables the production of pollen grains, and
consequently the reproduction of the plant, to be
controlled.

Such control proceeds via the production of
25 plants with one of their gametes totally sterile so as
to prevent self-fertilization.

So far, male sterility of plants, which is less
complex than female sterility, has been widely studied
but necessitates the use of genetic systems which are
30 relatively laborious to implement for commercial
production of hybrid seeds. One type of male sterility
which is highly used is cytoplasmic male sterility
which consists in producing:

- a female line whose sterile-male character-
35 istic is transmitted through the cytoplasm;
such a cytoplasm is called a "male-sterility-
inducing cytoplasm"; these "inducing
cytoplasms" are, for a given species, in
general discovered in the wild, or sometimes

observed in plants which result from interspecific crosses (cross-fertilization, protoplast fusion, etc.),

- 5 - a "sterility-maintaining" line whose cytoplasm is normal, and
- a fertility-restoring line if the seeds and/or the fruit of the hybrid plant are harvested.

10 In the female line (carrier of the sterility-inducing cytoplasm) all the pollen grains are killed. To multiply and improve this line it is therefore necessary to have a line which carries neither the inducing cytoplasm (which thus produces pollen grains)
15 nor the restoration gene. This line is termed "sterility-maintaining" because crossing with the female line gives an entirely female lineage.

 Restoration of the fertility is carried out in the hybrid by crossing the female parent (carrying the
20 sterile male cytoplasm) with the parent comprising a nuclear restoration gene (the restoring line), this cross enabling the production of fertile hybrid plants which will produce seeds by self-fertilization.

 In the case of sporophytic nuclear sterility,
25 systems have been described, for example, which make it possible to kill the mother cells of the microspores by means of an RNase, and, consequently, to obtain plants lacking in the male gametes. The fertility is restored when the line which no longer produces male gametes is
30 crossed with another line carrying an inhibitor of the RNase, the seeds resulting from this cross comprising both the cytotoxic gene and its inhibitor.

 As for the present invention, it proposes to produce plants with gametophytic male sterility, which
35 are incapable of producing pollen grains. It consists in using a promoter region which controls the expression, specifically in the microspores, of a gene encoding a cytotoxic molecule, while also having a

means permitting the controlled inhibition of this toxicity, in order to obtain a line of homozygous progenitor plants which are totally sterile as regards their male gametes, and then to obtain fertile hybrid
5 plants (which produce one viable pollen grain in two), thus capable of producing seeds, without having to resort to using a fertility restoration gene.

So far, a single gene which is expressed specifically in the microspore has been described, in
10 tobacco (Oldenholf et al., 1996). This gene does not have any homology with the Brassicaceae as results from a Southern Blot experiment on the genomic DNA of *Brassica oleracea* (data not shown).

A subject of the present invention is therefore
15 a nucleotide sequence for which it has been demonstrated that the corresponding gene is expressed specifically in the microspore; this nucleotide sequence corresponds to SEQ ID No. 3.

Consequently, a subject of the present
20 invention is a nucleotide sequence corresponding to all or part:

- a) of the sequence according to SEQ ID No. 3,
or
- b) of a sequence which hybridizes to the
25 sequence according to a), or
- c) of a sequence which has at least 80%
homology with a) or b).

In the context of the present invention, the most valuable part of this nucleotide sequence is the
30 promoter region which is defined as being the sequence preceding (on the 5' side) the translation start codon (ATG). However, at the current stage of knowledge about the nucleotide sequence according to SEQ ID No. 3, three ATGs have been shown: one at position 1965,
35 another at position 2085 and a third at position 2112. It would appear that the functional ATG is the one situated at position 2085. This is not confirmed however; it is the reason why the largest envisagable

promoter region concerning SEQ ID No. 3 stretches from nucleotide 1 to nucleotide 2111, and preferably from nucleotide 1 to nucleotide 2084.

5 This promoter region thus precedes, in the natural state, a coding (orf) sequence which is expressed specifically in the microspores, and in the case where this orf is replaced (by genetic manipulation) by another orf whose product is a cytotoxic molecule, the latter is capable of destroying
10 only said microspores.

A subject of the invention is therefore also cellular expression vectors, comprising a promoter sequence such as that described above, placed upstream of a DNA sequence encoding a cytotoxic product.

15 Advantageously, the cytotoxic product in question is a protease. Specifically, when the protease is expressed specifically in the microspores, it destroys all the proteins thereof, as a result of which the microspore cannot survive. Preferably, the protease
20 is a subtilisin, and in particular the BPN' subtilisin from *Bacillus amyloliquefaciens*. This BPN' subtilisin is part of the family of subtilisins which are found in many organisms and which are proteases known to cleave proteins at the level of serines.

25 It involves, therefore, introducing a vector in accordance with the invention into a bacterial strain capable of carrying out the transformation of plant cells, such as *Agrobacterium tumefaciens*. This may in particular be carried out by the method of infiltration
30 of *Arabidopsis thaliana* plants described by Bechtold et al., 1993. This technique consists in introducing the bacterium into the cells of the floral scapes by infiltration under vacuum. The plants are then bedded out under glass and their seeds harvested. About one
35 seed in a thousand gives rise to plants of which all the cells carry the transgene. The transformation of other plants, and in particular of rape, may be carried out through *Agrobacterium tumefaciens* and/or

Agrobacterium rhizogenes with the aid of various techniques, now conventional (transformation of foliar disks, of hypocotyls, of floral scapes etc.) which combine a phase of coculture of the bacterium with the
5 plant tissues, followed by the selection and by the regeneration of the transformed cells into whole plants. Other transformation techniques do not use this bacterium, but make it possible to transfer the cloned gene directly into cells or tissues (electroporation,
10 particle gun etc.), and to select and obtain transformed plants (review by Siemens and Schieder).

A subject of the present invention is also the cells of plants transformed with a vector in accordance with the invention and plants comprising said cells.

15 A subject of the invention is also plants with gametophytic male sterility with inducible fertility, comprising a gene encoding a male-gamete-specific cytotoxic product.

As indicated above, the present invention thus
20 enables the production of plants with gametophytic male sterility which inhibits any production of pollen grains. However, these plants, which are homozygous as regards their male sterility, may be obtained only after self-fertilization of plants which have
25 previously been transformed with a vector in accordance with the invention, i.e. which are hemizygous as regards their male sterility and in which the fertility of the pollen grains carrying the gametophytic sterility has been provisionally restored, so as to
30 allow them to carry out self-fertilization.

One means of producing plants which are homozygous for this gene would be to use gynogenesis, a technique which consists in regenerating doubled haploid plants from ovule or ovary culture. It
35 involves, in this case, obtaining the formation of a homozygous diploid plant from a female haploid gamete. Gynogenesis is applicable to a certain number of plant species, but production of a large number of plants

which are homozygous for the transgene in question is not envisagable by this technique, because it is tricky to use and its efficacy most often remains very poor.

The present invention also concerns a method
5 for producing plants with gametophytic male sterility with inducible fertility, comprising:

- the insertion into plants of line A of a gene whose expression product is cytotoxic for the microspores, and
- 10 - the production of plants which do not produce male gametes.

More particularly, the method for producing plants with gametophytic male sterility with inducible fertility in accordance with the invention comprises
15 the steps of:

- a) transformation of plants of a line A with a vector in accordance with the invention,
- b) induction of the fertility of the plants obtained in a) by inhibition of the cytotoxicity of the product,
- 20 c) self-fertilization of the fertile plants obtained in b),
- d) selection of the plants which do not produce male gametes, derived from c),
- 25 e) multiplication of the plants obtained in d) by repeating steps b) and c).

Thus, in step a) of the method above, a line A is transformed with a vector in accordance with the invention, i.e. comprising a microspore-specific
30 promoter sequence placed upstream of a gene encoding a cytotoxic product. The plants resulting from this transformation all comprise the DNA in question whose gene is expressed only in the microspores. However, at this stage, the plant being diploid at the time of the
35 transformation, it becomes heterozygous as regards its male sterility and is therefore capable, after transformation, of giving rise to microspores of which

the transgene, are thus destroyed by the cytotoxic product). A 50% production of the pollen is more than enough to give rise to seeds having the qualities of each of the crossed lines which it is specifically
5 desired to combine.

The present invention thus also concerns a method for producing hybrid plants, characterized in that it comprises crossing plants of line A, which have gametophytic male sterility as described above, with
10 plants of line B of agronomic value. It also concerns the seeds derived from the hybrid plants thus obtained.

Advantageously, the plants in accordance with the invention belong to the Brassicacea family; preferably, they are rape.

15 In addition, it should be pointed out that the promoter region in accordance with the invention may also be used in strategies of gene inactivation by utilization of mobile elements such as transposons and retrotransposons.

20 Specifically, this may be carried out with the aim of isolating plants which have a stable mutant genotype, and isolating a very large number of different, independent mutants.

It involves creating a chimeric sequence
25 consisting of a promoter region in accordance with the invention and of the sequence, all or in part, of a mobile element. The expression of this mobile element, which is reduced to the phase of development of the microspore, should make it possible to induce some
30 mutations into the genome of the pollen grains of the transformed plant. It is thus possible, in the lineage obtained from these pollen grains, to isolate individuals which no longer carry the transgene, but merely one or more mutations derived from transposition
35 phenomena. The principle is to bring about, using the abovementioned promoter region, activation of the transposition of these mobile elements for a very short time (microsporogenesis) in a multitude of gametic

cells and to eliminate in the following generation the plants which carry the transgene (i.e. the promoter region + the sequence which allows the activation of the transposition) so that the cycle does not start up again. It then involves investigating, in the lineage, and by various techniques, the plants for which the mobile elements have caused mutations by inserting themselves into genes. The study of these plants would make it possible, in particular, to understand the function of the mutated gene.

Among the mobile elements which can be used in this way, mention may be made of the retrotransposons of the type Tnt1, Tto1, Tnp-2, Tos10-17, Bsl, BARE-1, Ta-1, etc., or the transposons of the type Ac/Ds, Spm, Mu, etc.

Figure 1 illustrates the alignment of the sequences of the two cDNAs M3 (SEQ ID No. 1) and M3.21 (SEQ ID No. 2) derived from the screenings of the *Brassica napus* cv.Brutor microspore cDNA library. The start (ATG) and stop (TGA) codons of the putative coding sequence are underlined.

Figure 2 gives the nucleotide sequence of the clone BnM3.4 (SEQ ID No. 3) from which the M3 cDNA is thought to be derived. The ATG in bold (position 2085) is the one which has the highest probability of being the functional ATG. The ATG underlined in position 2112 is the one present in the M3.21 cDNA sequence. The ATG underlined in position 1965 is the first ATG encountered. The sequence preceding these ATGs is, consequently, taken to be the promoter region of the BnM3.4 gene.

Figure 3 illustrates the Northern Blot hybridization with the P³²-labeled M3 probe on total RNAs (10 µg per well) extracted from different rape tissues. A: buds of 0-2 mm (meiocytes); B: buds of 2-3 mm (mononucleated microspores); C: buds of 3-4 mm (binucleated microspores); D: buds greater than 4 mm (mature pollen grains); E: rape sepals; F: rape

pistils; **G**: buds of sterile male rape; **H**: full buds of rape.

Figure 4 illustrates the preparation of the 7152 bp pJD51 plasmid from the 5135 bp pAF1 plasmid (plasmid of origin: pBluescript SK-PROMEGA) and from the 5458 bp pBnB2 plasmid (plasmid of origin pBS SK-PROMEGA).

Figure 5 illustrates the preparation of the 19670 bp pJD101 plasmid from the 15400 bp pEC2 plasmid which is derived from the pDHB 321.1 plasmid (D. Bouchez, personal communication) and from the pJD51 plasmid (cf. Figure 2).

Figure 6 represents a scheme of selection of hybrid varieties of a plant (rape for example) which calls upon a system of gametophytic male sterility with induction of the fertility. SMGfi: gametophytic male sterility with inducible fertility; Induction F: induction of the fertility; AF: self-fertilization.

The invention is not limited to the sole description above; it will be better understood in the light of the following examples, which are given, however, purely as illustrations.

EXAMPLE 1: Demonstration of a microspore-specific promoter

The first step consisted in producing complementary DNA (cDNA) clones expressed specifically in the microspore of rape. For this, cDNAs were synthesized from rape microspore messenger RNAs (mRNA). In parallel, cDNAs were synthesized from floral bud mRNA from sterile male rape. The cDNAs coming from said floral buds were subtracted from the cDNAs derived from the mRNAs expressed in the microspore of rape. The molecules resulting from this subtraction were used in an experiment of differential hybridization of a microspore cDNA library, according to a technique similar to that presented by Atanassov et al. (1996).

One of these isolated clones, the M3 cDNA (SEQ ID No. 1), proved to be the representative of an mRNA which is specifically expressed in the microspore of rape. Another cDNA, named M3.21 (SEQ ID No. 2) was found by screening the library with the M3 cDNA. The sequences of these two cDNAs show 89% identity (Figure 1); they are clearly derived from a family of very close genes, which are expressed specifically in the microspore.

10 The M3 cDNA clone was used as a probe to screen a rape genomic DNA library sold by CLONTECH Laboratories, Inc., 4030 Fabian Way, Palo Alto, CA 94303-4607, USA; two clones (BnM3.4 and BnM3.2) corresponding to two different genes were isolated. The
15 M3 cDNA is thought to be derived from the BnM3.4 (SEQ ID No. 3) gene, because this gene carries an orf which is identical to the M3 cDNA (Figure 2). This gene has no intron. Sufficient experimental results lead to the thought that the M3.21 cDNA is not derived from the
20 second isolated gene (BnM3.2), which indeed carries a region corresponding to the M3.21 cDNA sequence, but to a third gene, which is very close to the BnM3.2 gene.

The promoter region of this gene is defined as being the sequence immediately upstream of the
25 translation start codon (ATG).

EXAMPLE 2: Verification of the specificity of the promoter of the BnM3.4 gene

A/ Northern Blot

A Northern Blot analysis was carried out with
30 10 µg of total RNA from sepals, pistil, whole buds, buds from sterile male plants, meiocytes, microspores, binucleated pollen grains and trinucleated pollen grains, hybridized with the M3 cDNA. A band of 1 kb corresponds to the transcript of the BnM3.4 gene, and
35 also to the M3.21 transcript, since they are very close sequences. These transcripts are present uniquely in the first two stages of male gametogenesis, whose

products are difficult to isolate perfectly experimentally (Figure 3).

The proteins deduced from these two cDNA clones are evidently very close and are rich in glycine and proline. They are identical to strictly no other protein in the databanks, but are certainly involved in the formation of the wall.

B/ Transformation with a chimeric gene

Different chimeric genes (i.e. consisting of the sequence encoding a known gene, preceded by the promoter region in accordance with the invention) were constructed in order to study the spatio-temporal specificity of the BnM3.4 promoter.

Figure 4 shows the construction of a bacterial vector pJD51, which combines a fragment of the BnM3.4 promoter with the sequence encoding the β -glucuronidase gene. The pAF1 plasmid containing the sequence encoding β -glucuronidase and the transcription termination sequence of the NOS gene from *Agrobacterium tumefaciens*, was digested with the enzymes BamHI and ClaI. The pBnB2 plasmid contains a 6 kb BamHI-BamHI fragment derived from the BnM3.4 genomic DNA clone, and in which the BnM3.4 gene is present. A fragment corresponding to the largest promoter region possible given the restriction sites (2056 bp) was isolated from the pBnB2 plasmid by a BamHI-NspV digestion, and inserted between the BamHI and ClaI (compatible with NspV) sites of the pAF1 plasmid.

The chimeric gene thus constructed was isolated by a NotI digestion of the pJD51 plasmid, so as to be cloned into a binary plasmid from *Agrobacterium tumefaciens*: pEC2 opened by the enzyme NotI (Figure 5).

The pJD101 binary plasmid containing the chimeric gene was introduced into the C58C1 strain (pMP90) of *Agrobacterium tumefaciens* (Koncz et al. 1986) by electroporation, and the transformants possessing pJD101 were selected on a medium containing kanamycin. One of these *Agrobacterium* transformants was

used to transform *Arabidopsis thaliana* (Wassilevskja
ecotype) by the method of infiltration of the floral
scapes described by Bechtold et al., 1993. The
transformed plants are selected using their resistance
5 to phosphinothrycin, which is conferred by a resistance
gene jointly inserted into the T-DNA.

Among these plants, certain show expression of
the β -glucuronidase specifically in the microspores
(demonstrated by a blue coloration when a
10 β -glucuronidase-specific substrate, X-Glu, is added).
No coloration is present in the adjacent tissues of the
anther, nor in the somatic tissues of the plant. In a
transformed plant which is hemizygous for the chimeric
gene, half the microspores produced are blue, because
15 only they contain the chimeric gene.

The specificity of expression conferred by this
2 kb promoter sequence is indeed restricted, within the
limits of the sensitivity of the technique, to a single
cell type, and from the microspore stage.

REFERENCES

- 5 Atanassov I et al. (1996) Plant Science 118, 185-194
- Bechtold N. et al. (1993) Comptes-Rendus de l'Académie
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- 10 Koncz et al. 1986) Molecular General Genetics 204,
383-396
- 15 Mariani et al. Nature 347 (1990) 737-741
- Oldenholf M.T. et al. (1996) Plant Molecular Biology
31, 213-225
- 20 Siemens and Schieder 1996. Plant Tissue Culture and
Biotechnology, 2, 66-75

WO 99/15678

- 1 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: INRA (INSTITUT NATIONAL DE
LA RECHERCHE AGRONOMIQUE)
(B) STREET: 147 RUE DE L'UNIVERSITE
(C) CITY: PARIS
(E) COUNTRY: FRANCE
(F) POSTAL CODE: 75007

(ii) TITLE OF THE INVENTION: Microspore-specific
promoter and method for producing hybrid plants

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version
#1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 497 base pairs
(B) TYPE: nucleotide
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: M3

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTTGGATCTT TCCATGACCC CTTCTTGACC GGCTATGTCA AGCTACATTG CTCCACCGTT	60
GTTGGATCTA CTTACCTCC TCCTTCACAG GCTCCTTTAC ATGCTCCTTC TTCACAGGCT	120
CCTTCACATG CTCCTTCACA TGCTCCTTCA CAGGCTCCTT TAAATGCTCT TTAAATGCT	180
CCTTTACATG CTCCTTTACA TGCTCCTTCA CAGGCCCTT CACAGGCCCC TTCACAGGCC	240
CCTTTACATG CTCCTTTACT GCCCCCTTCG CAGGCTCCTT CACCGGCTCA GTGATTTAGC	300
TATTTGATAG AATTACTCAA GTAATGATGC CCTAGGGAGT TTGAGTTTTT CTCGTGTTTT	360

AAAGTTTGT GTTTATTTTG AGAAAACCGT CTTTGGATTT TAACTTCACT TTGATTTTTT	420
CCCTTATACA ATTTAAATTT AGAGTTTACT TATTAATTTT ATAAATTAGA TGGTACTAAG	480
TTTTTATCAT AATAAAA	497

(2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 674 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 - (A) NAME/KEY: M3.21

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TCTTGCTATG ATTTTCTTCA TAAGATGTGT CACATCCAAA GTCACAGCAA CAGAACTAGA	60
GTCACTCAACT AACCAAGAGC TCTTCCTATC GCGGCACTTG CCTCGCTTTC ACCCCAAGCC	120
ACATTGGCCG TTCTGTGGCT CCGGAAAAGC CTTCCCTGCA GGCCACTTCC GACCAACTCC	180
GTTCCTCTG CCACAGGAAG TCACCAGATG CTTGTCCGAC AAGAAGGAGG TAGGTACATG	240
TTTGATGAT ATCGTTGAGA CTTTCTTCAC CAGGAAAGCC GTTATTGGAT CGGAATGTTG	300
CGCCGCGATC AAGAAGATGA ACAAAGATTG TGAGAAGACC GTCTTTGGAT CTTTCCATGA	360
CCCCTTCTTG ACAGGCTATG TCAAACTACA TTGCTCCACC GTTGTGATG CTACTTCACC	420
TCCTCCTTCA CATGCTCCTT CACAGGCTCC TTTACATGCT CCTTCACAGG CTCCTTTACA	480
TGCCCTTCA CAGGCTCCTT TACTGCCCCC TTCACAGCCT CTCCCACCGG CTCAGTGATT	540
TTAGCTATTT GTTAGAATTA TTCAAGTGTG GATGTCCTAG GGAGTTTATG GTTTTCTTG	600
TTTTAAAATT TTGTGTTTAT TTTGAGAAAA CCGTCTTTGG ATCTTAACTT CACTTTGATT	660
TTTTCCTTAT ACAA	674

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 2853 base pairs
 - (B) TYPE: nucleotide
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: BnM3.4

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GGATCCCACA AAGAAAACCG AAGAAGCAAA TGTTTCCTAC CTTCATAAAT ATATATTTGT	60
TTCAGCCTCA TCAATGTACA AACAATCCTT TAGCTCAATG GTATAAATGT TGTGTGTTAG	120
ATTTCATAA CCCGGGTTCG AGTCATAGAC TTGACACTTT TTCACACTTT TTAAGTGG	180
AACGCACATA TCGCTGACGT GTCGCATCAG GAGTGATGCA ACTGCTCTAT TATAATGTAG	240
ATTTAAAAGT GGAACCCACG TATCGCTGAC GTGTCGCATC AGGAGTGATG CAACTGCCAT	300
ATTATAACGT AGATTGACG TTATTCCTTT TTAAATCTTA ATAATAATAC CAGNGCTTTT	360
ACTTATTAAT TTTGNGCATN GTTATCATGG TTTATGCNCT CTTTTTTTTT GANCCGTTGA	420
TTGGTTTATG CTTATTGAA TGTNGCCNAC GTAAGAAATG AAGAACAATT TATATTGGA	480
GAAATATAA TTTAATATGT TCAATATATA GAGAAAATAT TATNCCTTGA TGTTACTGTA	540
TGGATGCGAG TAGAAGATCT TTGAATAATA TTTGAGAACT TGCCTTTTCT CAAAAAGTAA	600
AATATTTGAT ATGTAACCTA AGTTAACACA TGAAATTA AAAAAATTA AATCAAAATA	660
GAAAAAACTG ATAGTGATCT ACCCTTCAAC GTTTTGAAC TATTCTTGGT TCACCCCTA	720
AACCTCTAAG TTCACCAAAC AATAAAATTT CATTATTGCA TATTCTATAT CTTTLAGAAA	780
GTGAAACAAA ATATTATCAA GTTATATTAT GTTTTTCAAA TAAAAGATA AAAAATAAAT	840
AAAAATAAT AGTAGTTACA AAAAAAAA ATTAATATTT TTACCAGCGT CANAAAACAC	900
TAAAACCTAA ACCCTAAATA TTAACTTTT AGGTAAACCC TAAACCTTTG GATAAATCTT	960
AAACATTAAA CATTAAACA CTAAACCCTA AATCCTAAAC TCTAAACCCT TAAGTGTTTA	1020
AATGTTTAGT GTTTTGTATT TATAGTTTAG GATTATCCA AAGGTTTAAG GTTTACCCAA	1080
GAGTTTATGG TTTAGGGATT ATGACTTAGG ATTTAGTGT TTAGTGACGA CGTTCAAAGT	1140
ATTTTTTAA AATATTTTT TTTGTAACAA CTACTATTTT TATTTATTTT TTTACCTTTT	1200
TATATTAAA ACATAATATA ATTTAATACT CCATCTGTTT CATATTAAGT GTCATTGTAA	1260

CATTATTTTT TTGTTACAAA AAAATTGTCA CTTTAGAATT CCAATGCAAA ATTTATTTAT 1320
TTTTTCAGCTA AAATTAATTG CAAAGTGCAT TGATCTTATA AATAATTTTA TTTATCTCAA 1380
ATGCTATATT GGTCAAACAT GTGTAATTAA TAGAACTTA ATTATATTTT ATTTATTTTT 1440
TCTTAATCTG TGTA AAAATG TCAAAGTAAA ATTTATTTAG AACGAATTG AGTAATATTT 1500
TGTTTCATTT TTTAAAAGAT ATCGAATATG AAATAACACA ATTTTATTGT ATGATGAACC 1560
TAAAAATTCA TCCTAAGAAG GTGAACGCAA GAATAAGTCA ACGTTTTGGG GAAAGCTAAC 1620
TATGGCCCAA AGTCATCAAA ATCTTTCTTG TATTTATCAA AATCCTTACA AATTTAGTTA 1680
GAGTTAATAG ACCAAACACA TGATTATCAT CATATTAGAA TATTCTAAAA AATTACTAGC 1740
GAATAATTAA AATCTTCTT TTATTTATCA AAATCCTTAT AAAAATTAT TTATATATAC 1800
TAAAACAATT TTAATTA AAA GAAAATAAGG GACCATGGAT ACATAAAAAT ATATGTTATT 1860
TCTTAAGATA GTGATAATAT TAATATATAC CAGTCCATAT ATTTATCAAA ATAAATAATA 1920
TTTTTCGTAG TCCGATAATC ATTACTATAA ATTCATAAAA CCACATGTAG ATGTATATTT 1980
TATTTATATA TATATATATA AACCTAACG CCTTACCACT CGATAACCAT CAAAACTTTT 2040
CTTCTCGTTT CGCTAACTCA AGGCTTCGAA AAGTAAAAA AACAATGAAG AATGTCACAC 2100
TGTTCTTGC TATGATCCTC TTCTTAAGCT GTGTCACATC CAAAGTTACA GCAACAGAAC 2160
TAGAGTCATC AACTAACCA GAGCTCTTCC TATCGCGGCA CTTACCTCGC TTTCACCCCA 2220
AGCAACATTG GCCGTTCCGT GGCTCCGAA AAGCCTCCC TGCAGGCCAC TTCCGACTAA 2280
CTCCGTTCCA TCTGCCACAG GAAGTCACCA GATGCTTGAA CGACAAGAAG GAGGTAGGTA 2340
CATGTTTTAA TGATATCGCT GAGACTTTCT TCACCAGGAA AGCCGCTATT GGATCGGAAT 2400
GTTGCGCCGC GATCAAGAAG ATGAACAAAG ATTGTAGAA GACCGTCTTT GGATCTTTCC 2460
ATGACCCCTT CTGACCGGC TATGTCAAGC TACATTGCTC CACCGTTGTT GGATCTACTT 2520
CACCTCCTCC TTCACAGGCT CCTTTACATG CTCCTTCTC ACAGGCTCCT TCACATGCTC 2580
CTTCACATGC TCCTTCACAG GCTCCTTTAA ATGCTCCTT AAATGCTCCT TTACATGCTC 2640
CTTTACATGC TCCTTCACAG GCCCCTTCAC AGGCCCTTC ACAGGCCCTT TTACATGCTC 2700
CTTTACTGCC CCCTTCGAG GCTCCTTCAC CGGCTCAGT ATTTAGCTAT TTGATAGAAT 2760
TATTCAAGTA TTGATGTCCT AGGGAGTTTT AGTTTTTTC TTGTTTTAAA ATTTGTGTT 2820
TATTTTGAGA AAACCGTCTT TGGATTTTAA CTT 2853

CLAIMS

1. Nucleotide sequence corresponding to all or part:
 - 5 a) of the sequence according to SEQ ID No. 3, or
 - b) of a sequence which hybridizes to the sequence according to a), or
 - 10 c) of a sequence which has at least 80% homology with a) or b).
2. Nucleotide sequence according to Claim 1, corresponding to all or part:
 - 15 a) of the sequence which stretches from nucleotide 1 to nucleotide 2111, preferably from nucleotide 1 to nucleotide 2084 of SEQ ID No. 3, or
 - b) of a sequence which hybridizes to the sequence according to a), or
 - 20 c) of a sequence which has at least 80% homology with a) or b).
3. Cellular expression vector, comprising a sequence according to Claim 2, placed upstream of a DNA sequence encoding a cytotoxic product.
4. Vector according to Claim 3, characterized in
25 that the cytotoxic product is a protease and preferably a subtilisin.
5. Plant cells transformed with a vector according to Claim 3 or 4.
6. Plants comprising cells according to Claim 5.
- 30 7. Plants with gametophytic male sterility with inducible fertility, comprising a gene encoding a male-gamete-specific cytotoxic product.
8. Method for producing plants with gametophytic male sterility with inducible fertility, comprising:
35 - the insertion into plants of a line A of a gene whose expression product is cytotoxic for the microspores, and

- the production of plants which do not produce male gametes.

9. Method for producing plants with gametophytic male sterility with inducible fertility according to

5 Claim 8, comprising the steps of:

- a) transformation of plants of a line A with a vector according to Claim 3 or 4,
- b) induction of the fertility of the plants obtained in a) by inhibition of the
10 cytotoxicity of the product,
- c) self-fertilization of the fertile plants obtained in b),
- d) selection of the plants which do not produce male gametes, derived from c),
- 15 e) multiplication of the plants obtained in d) by reproduction of steps b) and c).

10. Method for producing plants according to Claim 8 or 9, characterized in that, when the cytotoxic product is a subtilisin, the induction of the fertility
20 consists in applying to the plant an insecticide molecule of the fluorophosphate family.

11. Seeds derived from the hybrid plants obtained by crossing plants of line A, which have gametophytic male sterility with inducible fertility, according to
25 Claim 7, or as obtained by using the method according to one of Claims 8 to 10, with plants of line B of agronomic value.

12. Plants according to Claim 7, or obtained by using a method according to any one of Claims 8 to 10,
30 characterized in that they belong to the Brassicacea family and preferably in that they are rape.

M3 80
 M3.21 TCTTGCCTATG ATTTTCTTCA TAAGATGTCT CACATCCAAA GTCACAGCAA CAGAACTAGA GTCATCAACT AACCAAGAGC

 M3 160
 M3.21 TCTTCCCTATC GCGGCACCTTG CCTCGCTTTC ACCCCAAGCC ACATTGGCCG TTCTGTGGCT CCGGAAAGC CTTCCCTGCA

 M3 240
 M3.21 GGGCACTTCC GACCAACTCC GTTCCATCTG CCACAGGAAG TCACACAGATG CTGTGTCGAC AAGAAGGAGG TAGGTACATG

 M3 320
 M3.21 TTTTGTATGAT ATCGTTGAGA CTTTCTTTCAC CAGGAAGGCC GTTATTTGGAT CGGAATGTTG CCGCCGCGATC AAGAAGATGA

 M3 400
 M3.21 TTTGGAT CTTTCCATGA CCCCCTTCTTAC ACCGGCTATG TCAAGCTACA TTGCTCCACC
 M3.21 ACAAGAGATG TGAGAGAGCC GTCTTTGGAT CTTTCCATGA CCCCCTTCTTAC ACCGGCTATG TCAAGCTACA TTGCTCCACC

 M3 480
 M3.21 GTTGTGGAT CTACTTCACC TCCTCCTTCA CAGGCTCCTT TACATGCTCC TTTCTCACAG GCTCCTTCAC ATGCTCCTTC
 M3.21 GTTGTGGAT CTACTTCACC TCCTCCTTCA CAGGCTCCTT TACATGCTCC TTTCTCACAG GCTCCTTCAC ATGCTCCTTC

 M3 560
 M3.21 ACATGCTCCT TCACAGGCTC CTTTAAATGC TCTTTTAAAT GCTCCTTTAC ATGCTCCTTT ACATGCTCCT TCACAGGCTC
 M3.21 ACATGCTCCT TCACAGGCTC CTTTAAATGC TCTTTTAAAT GCTCCTTTAC ATGCTCCTTT ACATGCTCCT TCACAGGCTC

 M3 640
 M3.21 CTTTCACAGGC CCGTTTCACAG GCGCCCTTTAC ATGCTCCTTT ACTGCCCTT TCGCAGGCTC CTTTCACCGGC TCAGTGA-TT
 M3.21 CTTTCACAGGC TCCCTTACAT GCGCCCTTAC AGGCTCCTTT ACTGCCCTT TCGCAGGCTC TCCACCGGC TCAGTGA-TT

 M3 720
 M3.21 TAGCTATTG ATAGAATTAC TCAAGTAATG ATGCCCTAGG GAGTTTGGT TTTTCTCTG TTTTAAAGTT TTGTGTTTAT
 M3.21 TAGCTATTG TTAGAATTAT TCAGATGTTG ATGCTCCTAGG GAGTTTGGT TTTTCTCTG TTTTAAAGTT TTGTGTTTAT

 M3 800
 M3.21 TTTTCAGAAA CCGTCTTTGG ATTTTAACTT CACTTTGAT TTTTCCCTTA TACAATTAA ATTTAGAGTT TACTTATTAA
 M3.21 TTTTCAGAAA CCGTCTTTGG ATCTTAACTT CACTTTGAT TTTTCCCTTA TACA... ..

 M3 841
 M3.21 TTTTATAAAT TAGATGGTAC TAAGTTTATA TCATAATAA A
 M3.21

FIGURE 1

FIGURE 2

1 GGATCCCACA AAGAAAACCG AAGAAGCAAA TGTTTCCTAC CTTCATAAAT
51 ATATATTTGT TTCAGCCTCA TCAATGTACA AACAATCCTT TAGCTCAATG
101 GTATAAATGT TGTTGTTTGTG ATTTCAATAA CCCGGGTTTCG AGTCATAGAC
151 TTGACACTTT TTCACACTTT TTAAGAGTGG AACGCACATA TCGCTGACGT
201 GTCGCATCAG GAGTGATGCA ACTGCTCTAT TATAATGTAG ATTTAAAAGT
251 GGAACCCACG TATCGCTGAC GTGTCGCATC AGGAGTGATG CAACTGCCAT
301 ATTATAACGT AGATTTGACG TTATTCCTTT TTAATCTTA ATAATAATAC
351 CAGNGCTTTT ACTTATTAAT TTTGNGCATN GTTATCATGG TTTATGCNCT
401 CTTTTTTTTT GANCCGTTGA TTGGTTTATG CTTATTTGAA TGTNGCCNAC
451 GTAAGAAATG AAGAACAATT TATATTTGGA GAAATATATA TTTAATATGT
501 TCAATATATA GAGAAAATAT TATNCCTTGA TGTTACTGTA TGGATGCGAG
551 TAGAAGATCT TTGAATAATA TTTGAGAACT TGCCTTTTCT CAAAAAGTAA
601 AATATTTGAT ATGTAACCTTA AGTTAACACA TGAAAATTAA AAAAAATTA
651 AATCAAAATA GAAAAAATG ATAGTGATCT ACCCTTCAAC GTTTTGAACCT
701 TATTCTTGGT TCACCCCTA AACCTCTAAG TTCACCAAAC AATAAAATTT
751 CATTATTGCA TATTCTATAT CTTTATAGAA GTGAAACAAA ATATTATCAA
801 GTTATATTAT GTTTTTCAAA TAAAAAGATA AAAAATAAAT AAAAAATAAT
851 AGTAGTTACA AAAAAAAAAA ATTAATATTT TTACCAGCGT CANAAAAACAC
901 TAAACCTAA ACCCTAAATA TTAACTTTT AGGTAAACCC TAAACCTTTG
951 GATAAATCTT AAACATTAAC CATTAAACA CTAAACCTA AATCCTAAAC
1001 TCTAAACCTT TAAGTGTTTA AATGTTTAGT GTTTTGTATT TATAGTTTAG
1051 GATTTATCCA AAGGTTTAAG GTTTACCCAA GAGTTTATGG TTTAGGGATT
1101 ATGACTTAGG ATTTAGTGTT TTAGTGACGA CGTCAAAGT ATTTTTTAAA
1151 AAATATTTTT TTTGTAACAA CTACTATTTT TATTTATTTT TTTACCTTTT
1201 TATATTAAAA ACATAATATA ATTTAATACT CCATCTGTTT CATATTAAGT
1251 GTCATTGTAA CATTATTTTT TTGTTACAAA AAAATTGTCA CTTTAGAATT

FIGURE 2 (continued)

1301 CCAATGCAAA ATTTATTTAT TTTTCAGCTA AAATTAATTG CAAAGTGCAT
1351 TGATCTTATA AATAATTTTA TTTATCTCAA ATGCTATATT GGTCAAACAT
1401 GTGTAATTAA TAGAACTTA ATTATATTC ATTTATTTTT TCTTAATCTG
1451 TGTAAAAATG TCAAAGTAAA ATTTATTTAG AAACGAATTG AGTAATATTT
1501 TGTTTCATTT TTTAAAAGAT ATCGAATATG AAATAACACA ATTTTATTGT
1551 ATGATGAACC TAAAAATTCA TCCTAAGAAG GTGAACGCAA GAATAAGTCA
1601 ACGTTTTGGG GAAAGCTAAC TATGGCCCAA AGTCATCAAA ATCTTTCTTG
1651 TATTTATCAA AATCCTTACA AATTTAGTTA GAGTTAATAG ACCAAACACA
1701 TGATTATCAT CATATTAGAA TATTCTAAAA AATTACTAGC GAATAATTAA
1751 AATCTTTCTT TTATTTATCA AAATCCTTAT AAAAAGTTAT TTATATATAC
1801 TAAACAATT TTAATTAAAA GAAAATAAGG GACCATGGAT ACATAAAAAAT
1851 ATATGTTATT TCTTAAGATA GTGATAATAT TAATATATAC CAGTCCATAT
1901 ATTTATCAAA ATAAATAATA TTTTTCGTAG TCCGATAATC ATTACTATAA
1951 ATTCATAAAA CCACATGTAG ATGTATATTT TATTTATATA TATATATATA
2001 AACCCTAACG CCTTACCACT CGATAACCAT CAAAAGTTTT CTTCTCGTTT
2051 CGCTAACTCA AGGCTTCGAA AAGTAAAAAA AACAATGAAG AATGTCACAC
2101 TTGTTCTTGC TATGATCCTC TTCTTAAGCT GTGTCACATC CAAAGTTACA
2151 GCAACAGAAC TAGAGTCATC AACTAACCAA GAGCTCTTCC TATCGCGGCA
2201 CTTACCTCGC TTTCACCCCA AGCAACATTG GCCGTTCCGT GGCTCCGGAA
2251 AAGCCTTCCC TGCAGGCCAC TTCCGACTAA CTCCGTTCCA TCTGCCACAG
2301 GAAGTCACCA GATGCTTGAA CGACAAGAAG GAGGTAGGTA CATGTTTTAA
2351 TGATATCGCT GAGACTTTCT TCACCAGGAA AGCCGCTATT GGATCGGAAT
2401 GTTGCGCCGC GATCAAGAAG ATGAACAAAG ATTGTGAGAA GACCGTCTTT
M3 TTT
2451 GGATCTTTCC ATGACCCCTT CTTGACCGGC TATGTCAAGC TACATTGCTC
M3 GGATCTTTCC ATGACCCCTT CTTGACCGGC TATGTCAAGC TACATTGCTC
2501 CACCGTTGTT GGATCTACTT CACCTCCTCC TTCACAGGCT CCTTTACATG
M3 CACCGTTGTT GGATCTACTT CACCTCCTCC TTCACAGGCT CCTTTACATG

2551 CTCCTTCTTC ACAGGCTCCT TCACATGCTC CTTACATGC TCCTTCACAG
M3 CTCCTTCTTC ACAGGCTCCT TCACATGCTC CTTACATGC TCCTTCACAG

2601 GTCCTTTAA ATGCTCCTTT AAATGCTCCT TTACATGCTC CTTTACATGC
M3 GTCCTTTAA ATGCTCCTTT AAATGCTCCT TTACATGCTC CTTTACATGC

2651 TCCTTCACAG GCCCCTTCAC AGGCCCTTC ACAGGCCCTT TTACATGCTC
M3 TCCTTCACAG GCCCCTTCAC AGGCCCTTC ACAGGCCCTT TTACATGCTC

2701 CTTTACTGCC CCCTTCGCAG GTCCTTCAC CGGCTCAGTG ATTTAGCTAT
M3 CTTTACTGCC CCCTTCGCAG GTCCTTCAC CGGCTCAGTG ATTTAGCTAT

2751 TTGATAGAAT TATTCAAGTA TTGATGTCCT AGGGAGTTTT AGTTTTTTTC
M3 TTGATAGAAT TACTCAAGTA ATGATGCCCT AGGGAGTTTG AGTTTTTCTC

2801 TTGTTTTAAA ATTTTGTGTT TATTTTGAGA AAACCGTCTT TGGATTTTAA
M3 GTGTTTTAAA GTTTTGTGTT TATTTTGAGA AAACCGTCTT TGGATTTTAA

2851 CTT
M3 CTT

FIGURE 2 (continued)

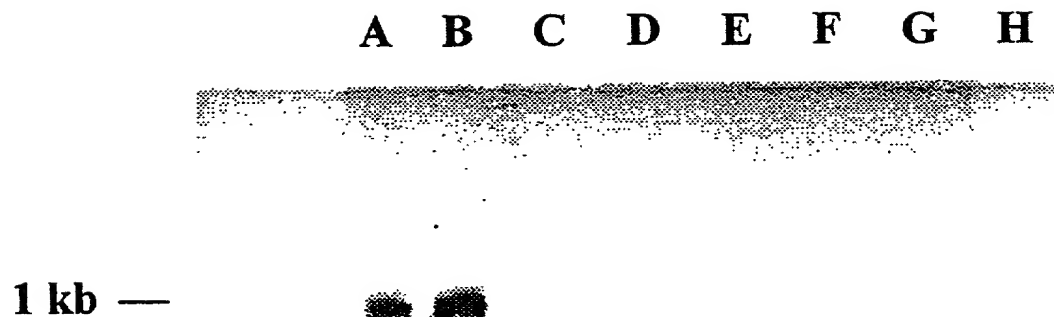


FIGURE 3

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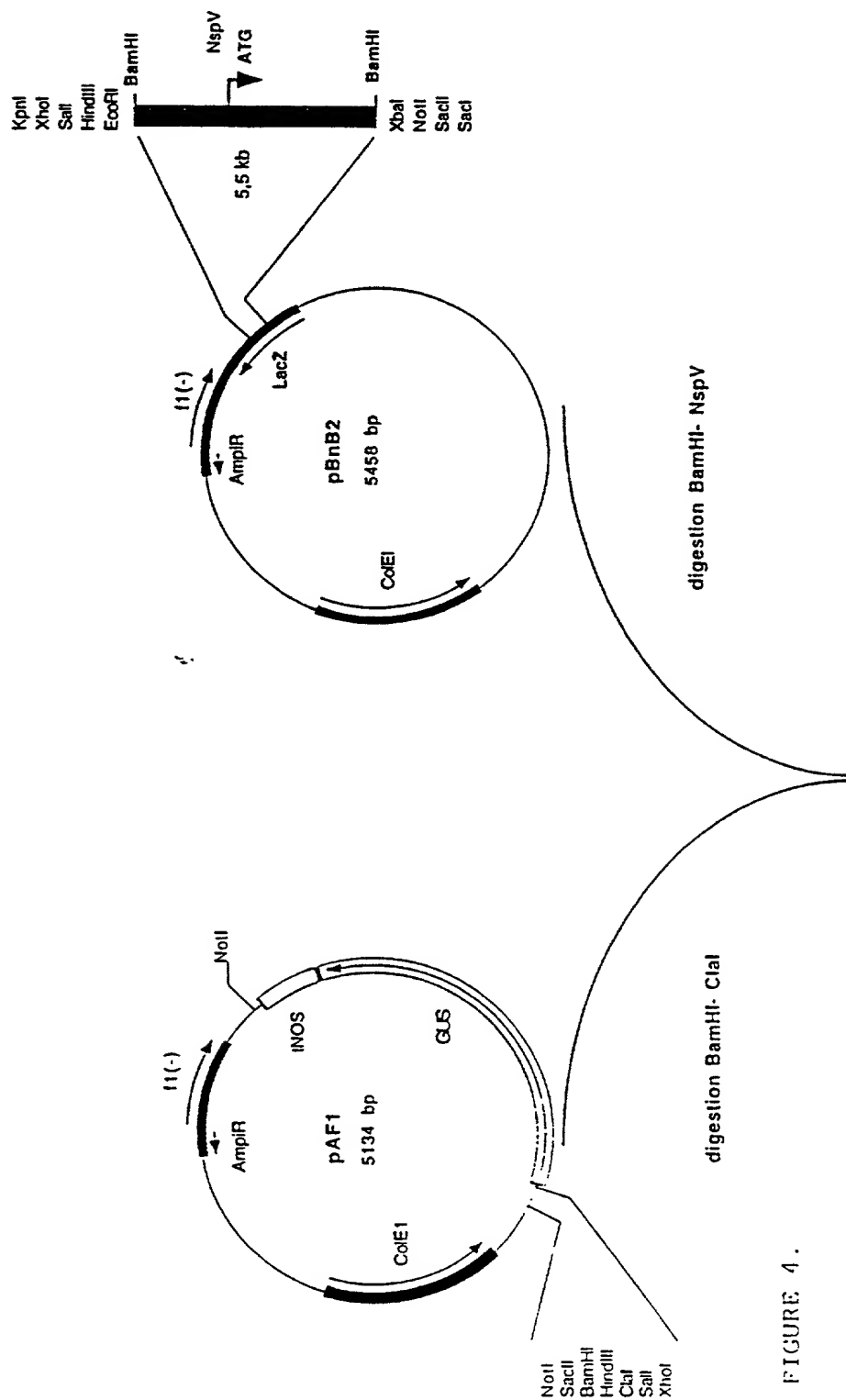


FIGURE 4.

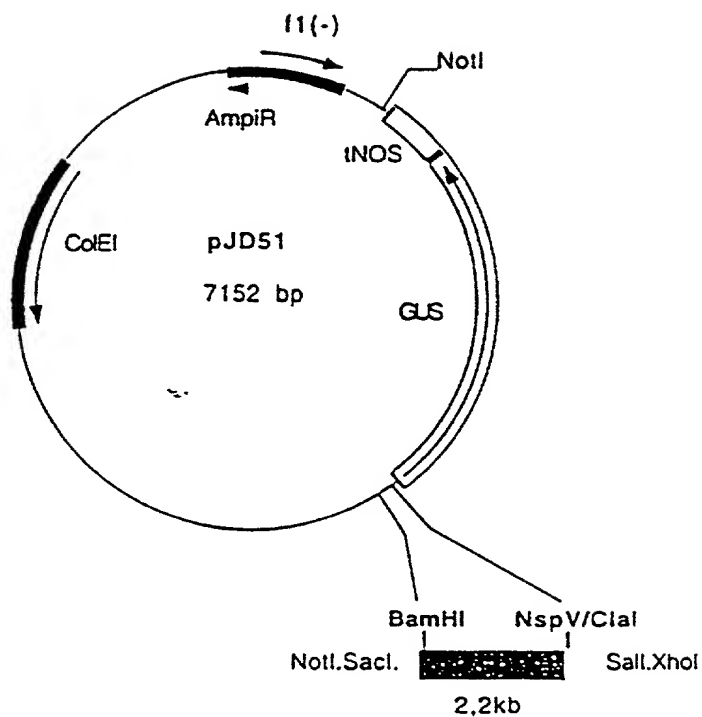


FIGURE 4 (continued)

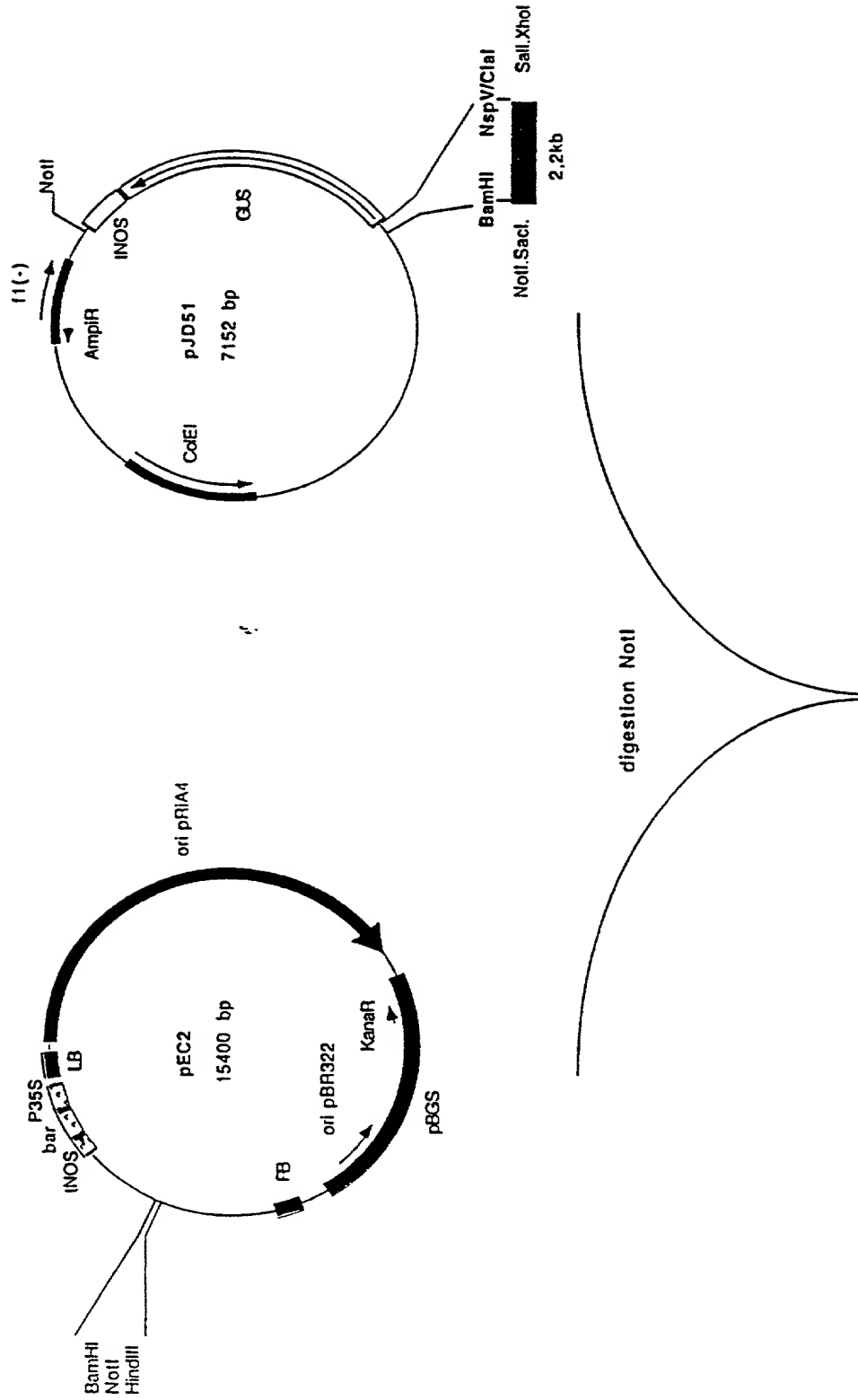


FIGURE 5.

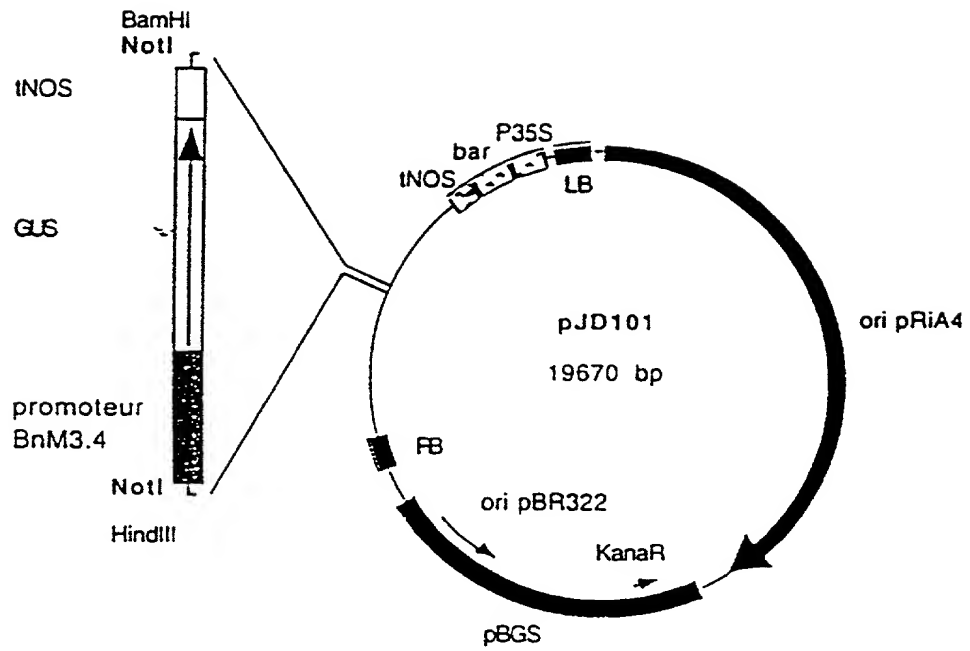


FIGURE 5 (continued)

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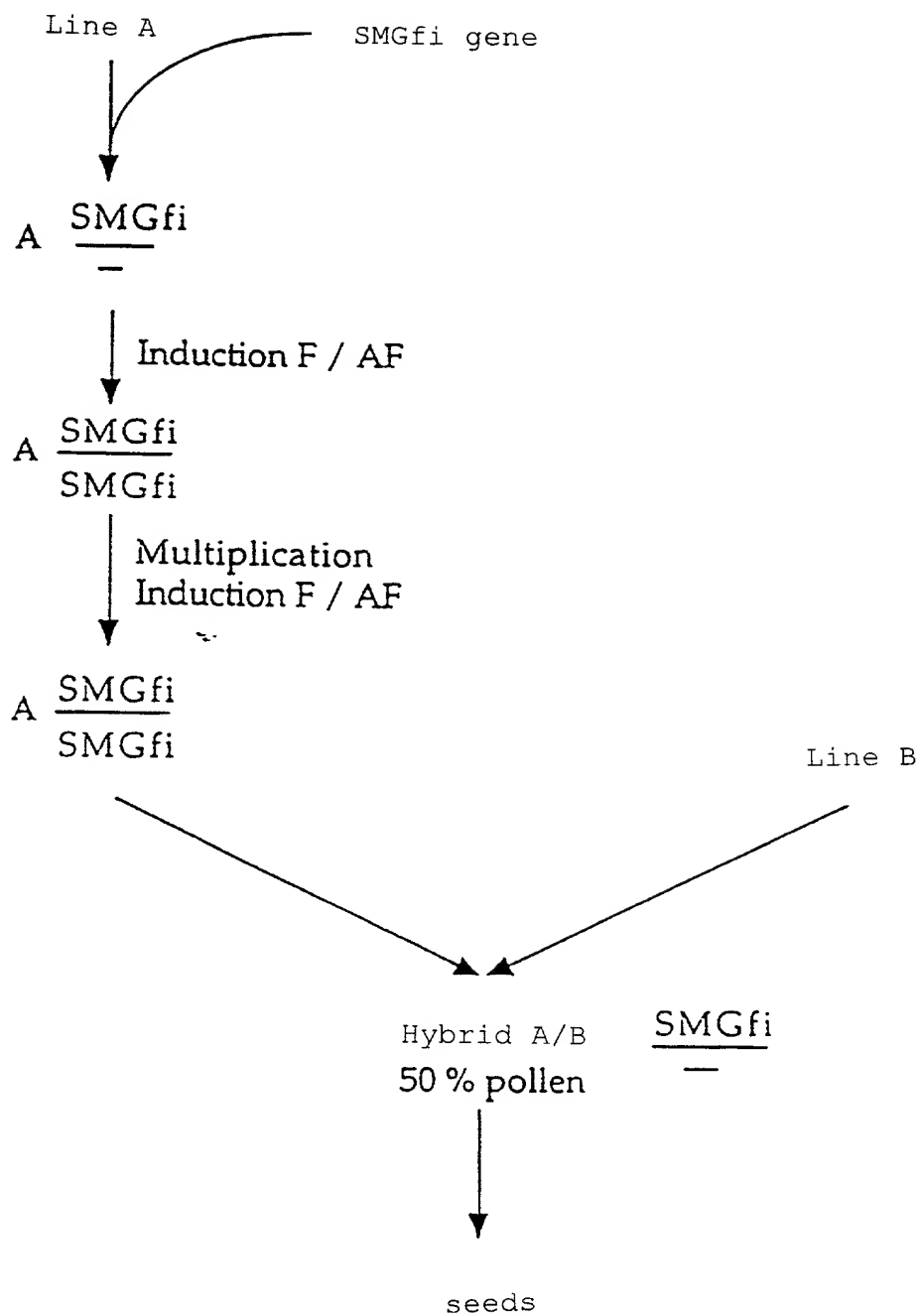


FIGURE 6

DECLARATION AND POWER OF ATTORNEY

#3

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled.

MICROSPORE-SPECIFIC PROMOTER AND METHOD FOR PRODUCING HYBRID PLANTS

the specification of which is attached hereto unless the following box is checked:

☒ was filed on SEPTEMBER 23, 1998 as United States Application Number or PCT International Application Number PCT/FR98/02042 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
97/11812	FRANCE	23/SEPTEMBER/1997	YES

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below

APPLICATION NO.	FILING DATE

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application

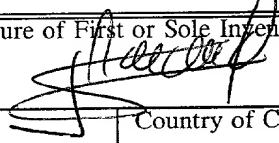
APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

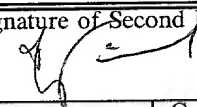
I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.


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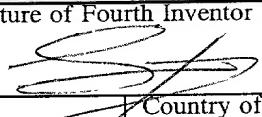
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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Residence Address	Country of Citizenship	
Post Office Address		